Mosquito surveillance, 2005

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Testing at CVEC

- RT-PCR: robotic RNA extraction using ABI 6700 followed by RT-PCR with TaqMan platform using a multiplex system testing simultaneously for WEEV, SLEV and WNV
- Rapid turn-around-time: "in by Wed out by Fri" paradigm with reporting on Friday.
- Testing not done for CEV or other viruses during 2005 to increase throughput, retain sensitivity and limit cost. *Aedes* and *Culiseta* saved for testing this winter.
- Confirmation done on some local testing.

Number of mosquito pools submitted to CVEC for testing

Agency	Total
Alameda County MAD	398
Alameda County VCSD	33
Antelope Valley MVCD	56
Butte County MVCD	63
Coachella Valley MVCD	2789
Consolidated MAD	323
Contra Costa MVCD	423
Delta VCD	100
El Dorado County Environmental Management	1
El Dorado Vector Control	3
Fresno MVCD	38
Fresno Westside MAD	71
Glenn County MVCD	47
Greater LA County VCD	2758
Imperial County Health Dept	163
Kern MVCD	1579
Kings MAD	62
Lake County VCD	359
Long Beach VCP	422
Los Angeles County West VCD	441
Madera County MVCD	22
Marin-Sonoma MVCD	28
Merced County MAD	619
Nevada County Dept of Agriculture	2

Agency	Total
Northwest MVCD	479
Orange County VCD	2743
Owens Valley MAP	127
Placer MAD	268
Presidio Trust	12
Riverside County Environ Health	429
Sacramento-Yolo MVCD*	2448
San Bernardino County VCP	465
San Diego Dept Envl Health	142
San Gabriel Valley MVCD	2
San Joaquin County MVCD	98
San Luis Obispo County Health Dept	48
San Mateo County MAD	141
Santa Barbara Coastal VCD	399
Santa Clara County VCD	3
Santa Cruz County MVCD	18
Shasta MVCD	52
Sutter-Yuba MVCD	421
Tehama County MVCD	3
Turlock MVCD	1317
Ventura County Environ Health Dept	45
West Side MVCD	278
West Valley MVCD	57
Grand Total	20795

*Includes pools by RT-PCR at Sacramento PHL

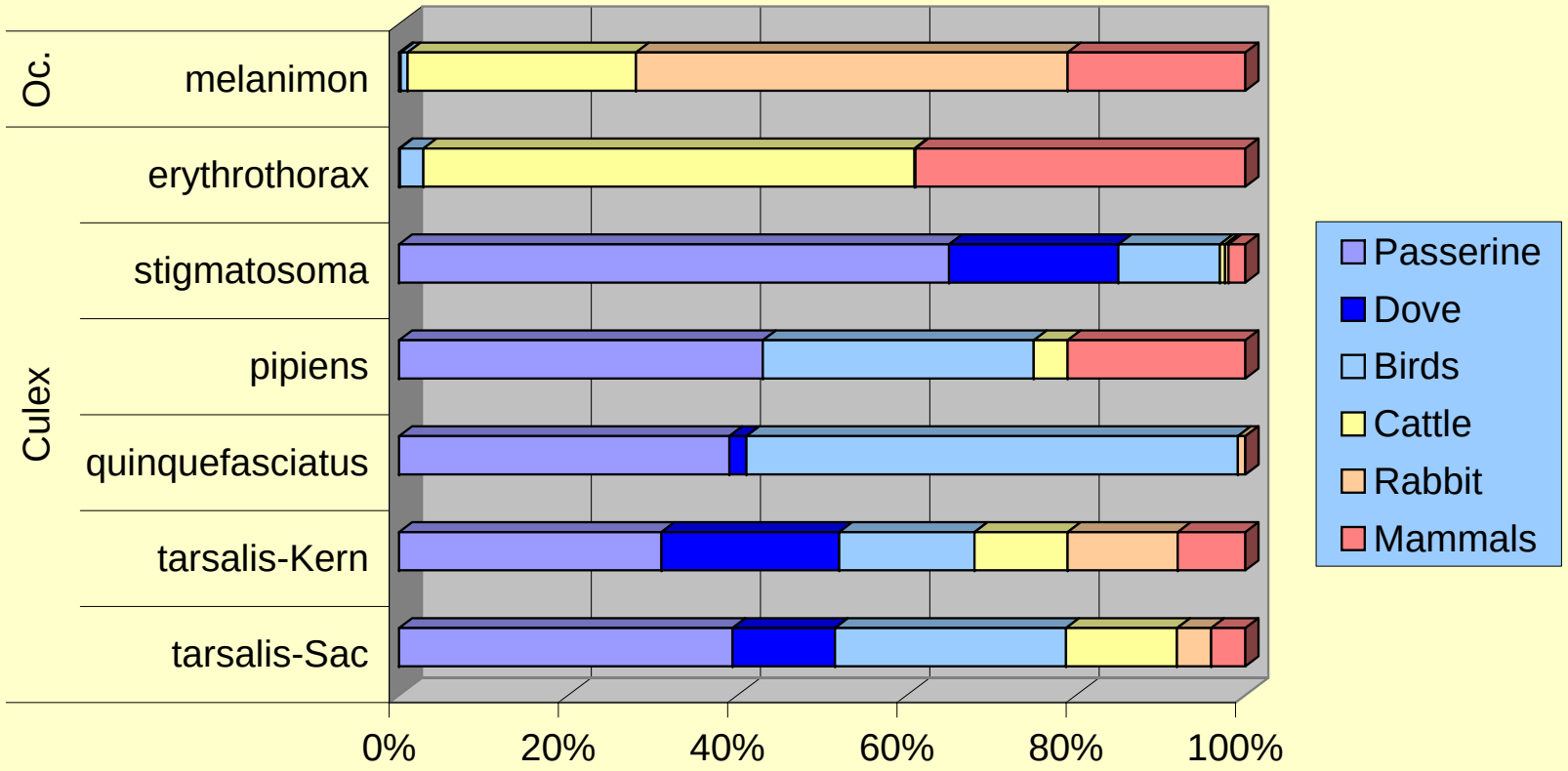
Species	Total pools	Number tested	WNV positive	MIR/ 1,000	WEEV positive	MIR/ 1,000
Ae vexans	134	4,572	0	0.00	0	
Ae washinoi	1	26	0	0.00	0	
An franciscanus	29	966	0	0.00	0	
An freeborni	90	3,001	1	0.33	0	
An hermsi	97	2,647	0	0.00	0	
An phorophora	2	44	0	0.00	0	
An punctipennis	1	50	0	0.00	0	
Cq perturbans	2	62	0	0.00	0	
Cs incidens	431	11,627	0	0.00	0	
Cs inornata	148	3,919	0	0.00	0	
Cs particeps	24	454	0	0.00	0	
Cx apicalis	1	5	0	0.00	0	
Cx erraticus	3	76	0	0.00	0	
Cx erythrothorax	2,088	91,165	26	0.29	0	
Cx pip/quinq	4	200	0	0.00	0	
Cx pipiens	2,519	68,743	240	3.49	0	
Cx quinquefasciatus	6,054	211,330	471	2.23	0	
Cx restuans	39	1,011	0	0.00	0	
Cx species	2	25	0	0.00	0	
Cx squamiger	1	50	0	0.00	0	
Cx stigmatosoma	596	12,849	33	2.57	0	
Cx tarsalis	7,688	303,832	458	1.51	51	0.17
Cx thriambus	98	3,306	7	2.12	0	
NONE GIVEN	4	48	0	0.00	0	
Oc dorsalis	22	840	0	0.00	0	
Oc fitchii	1	19	0	0.00	0	
Oc increpitus	5	173	0	0.00	0	
Oc melaminon	5	7	0	0.00	0	
Oc melanimon	605	21,835	0	0.00	0	
Oc nigromaculis	2	59	0	0.00	0	
Oc pullatus	1	31	0	0.00	0	
Oc sierrensis	23	675	0	0.00	0	
Oc squamiger	6	171	0	0.00	0	
Oc sticticus	6	230	0	0.00	0	
Oc taeniorhynchus	8	306	0	0.00	0	
Oc washinoi	49	1,871	0	0.00	0	
Ps columbiae	6	252	0	0.00	0	
Totals	20,795	746,477	1236	1.66	51	0.07

Summary of submissions and test results for 2005 by species [as submitted]

Conclusions

1. Only bird-feeding *Culex* infected with WNV
2. No *Ochlerotatus Aedes* infected – i.e, no mammal cycle?
3. *Culex* must be infecting humans and horses
4. Only *tarsalis* infected with WEEV in Imperial, Coachella and Kern

Host selection patterns of some California mosquitoes

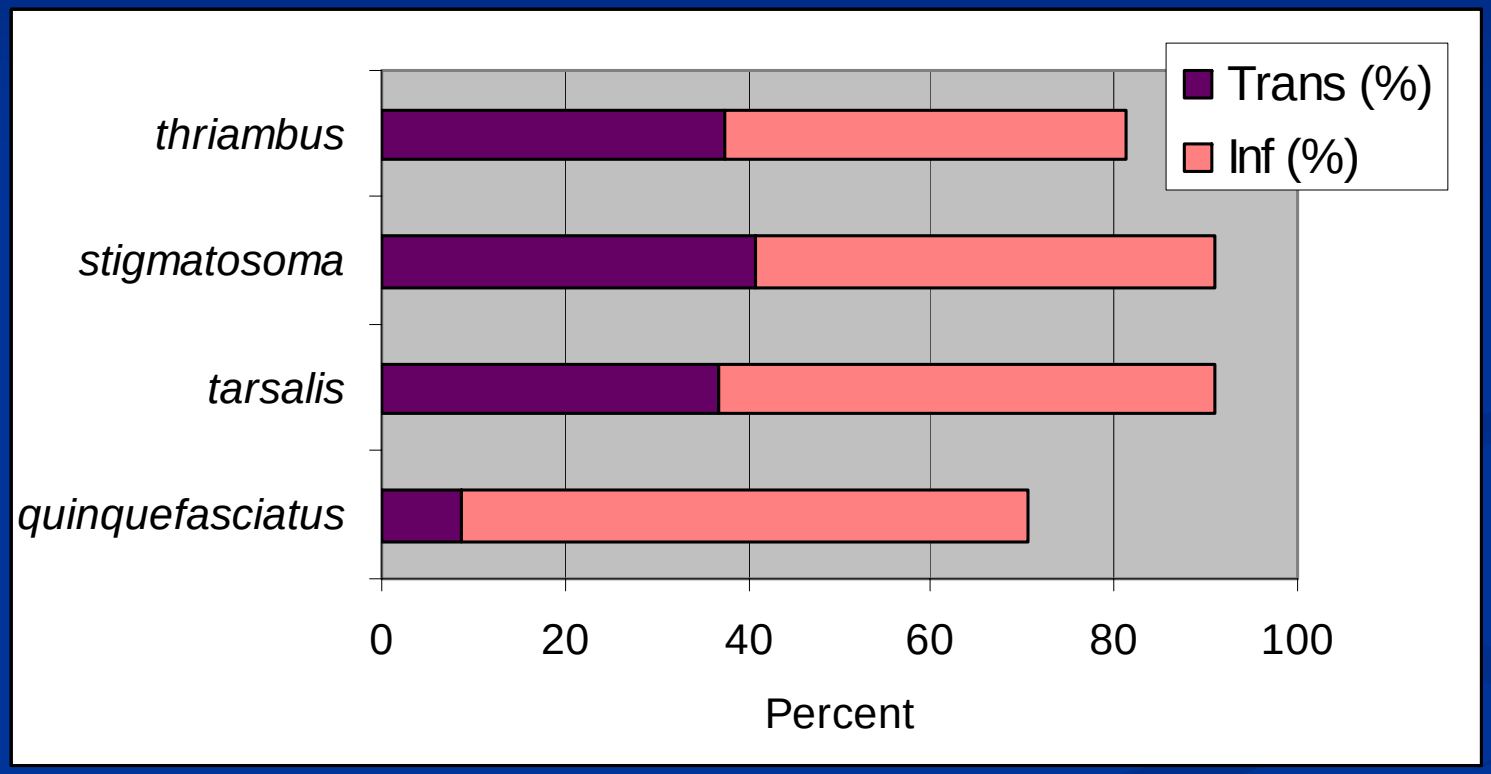


Data from: Reeves. 1990. Epidemiology and Control of Mosquito-borne Arboviruses in California, 1943-1987. Calif. Mosq. Vector Control Assoc.

MIRs during summer transmission season, Jul – Sep 2005

Culex	Pools	Total	WNV pos	MIR/1000
Cx. erythrothorax	871	38,460	16	0.42
Cx. pipiens	1,839	50,097	231	4.61
Cx. quinquefasciatus	2,484	83,771	405	4.83
Cx. stigmatosoma	260	5,157	30	5.82
Cx. tarsalis	3,355	120,701	390	3.23
Cx. thriambus	54	1,728	7	4.05
Total	8,863	299,914	1,079	3.60

Vector competence of *Culex* species tested from California during 2005

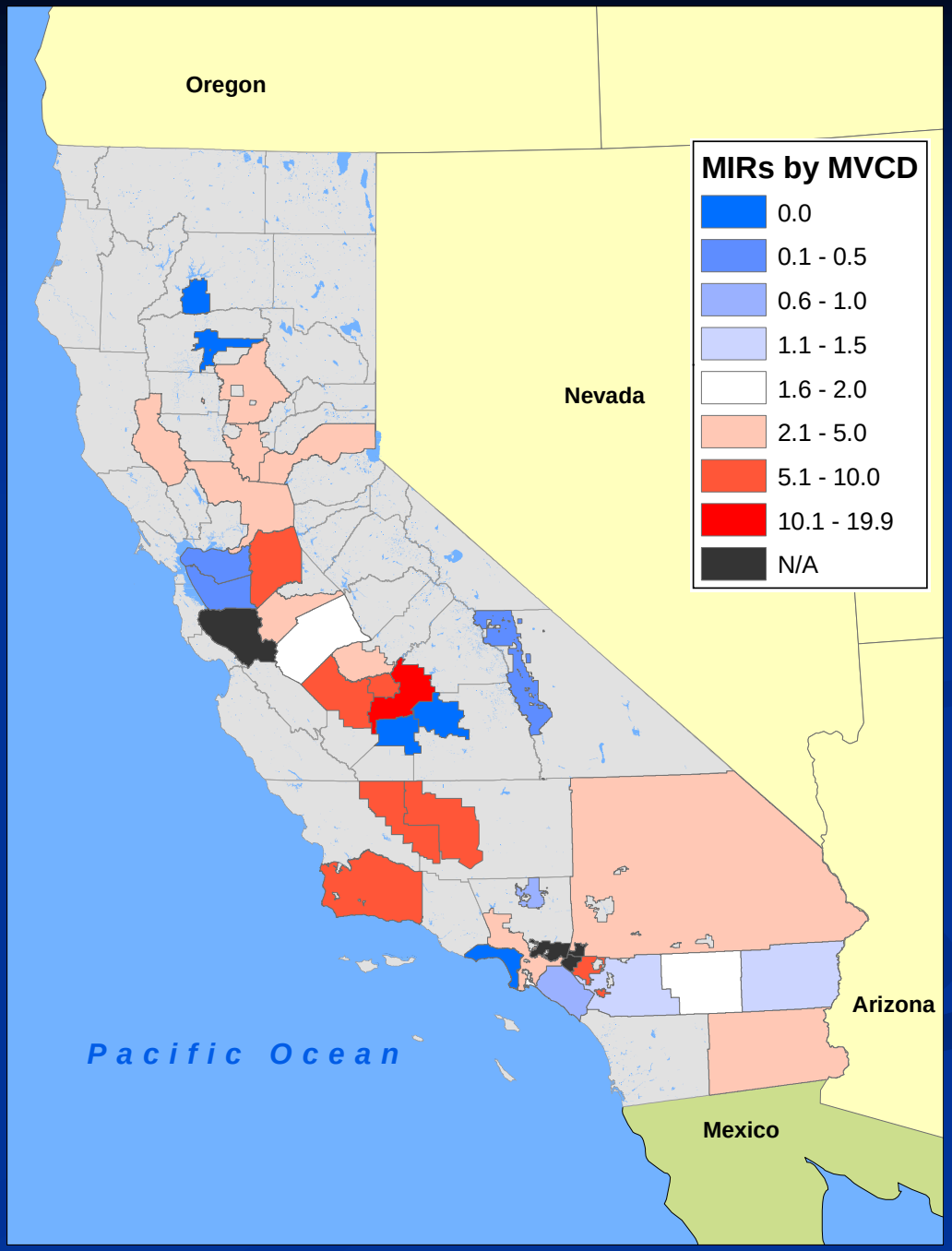


Data summarizes 1 – 4 exps with each species; dose >6 $\log_{10}$ PFU/mL, EIP 2 wks at 26C

Cx. tarsalis, summer 2005

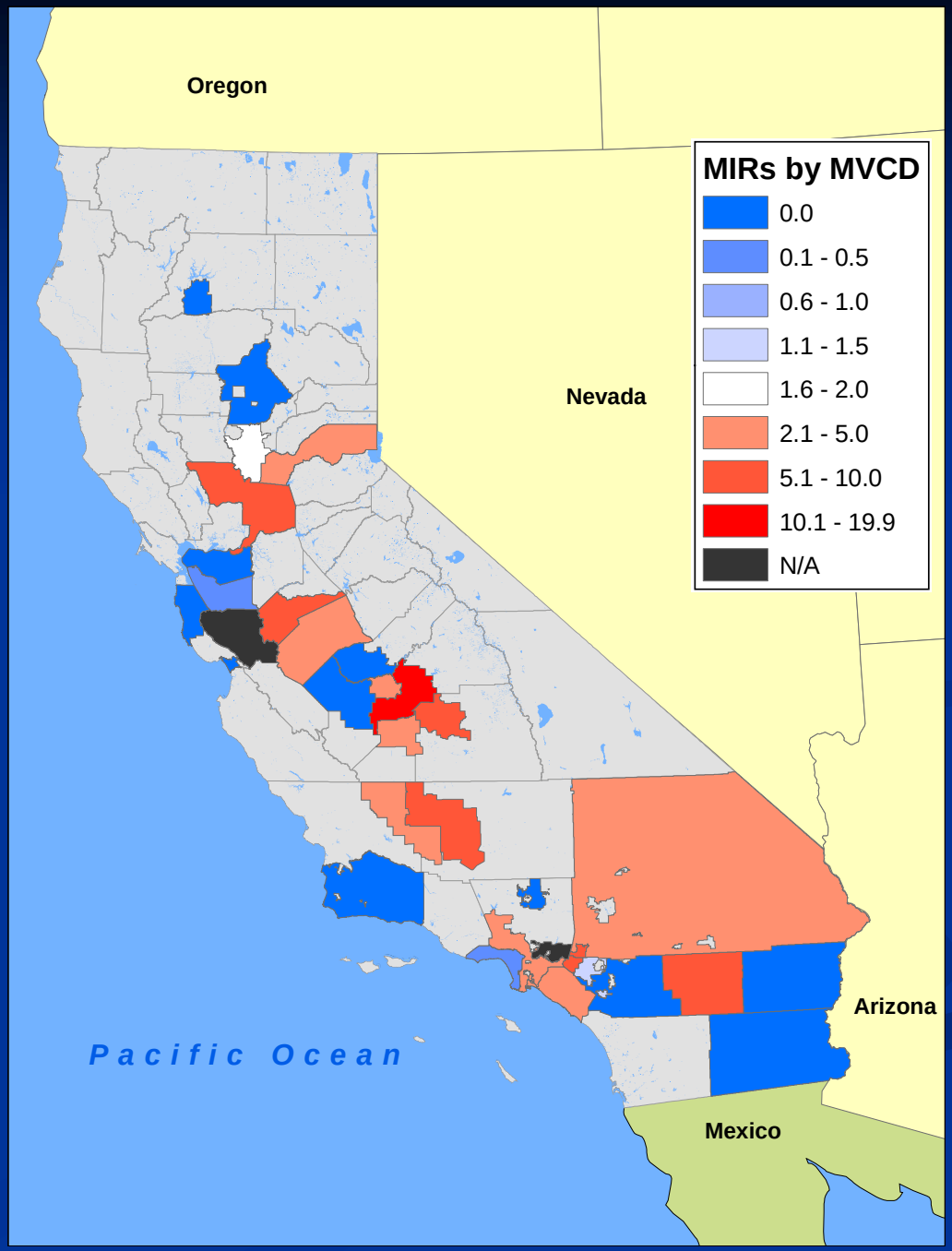
Note: MIRs >5/1,000 frequently are associated with human and/or equine infection.

N/A – not available, mosquitoes tested locally

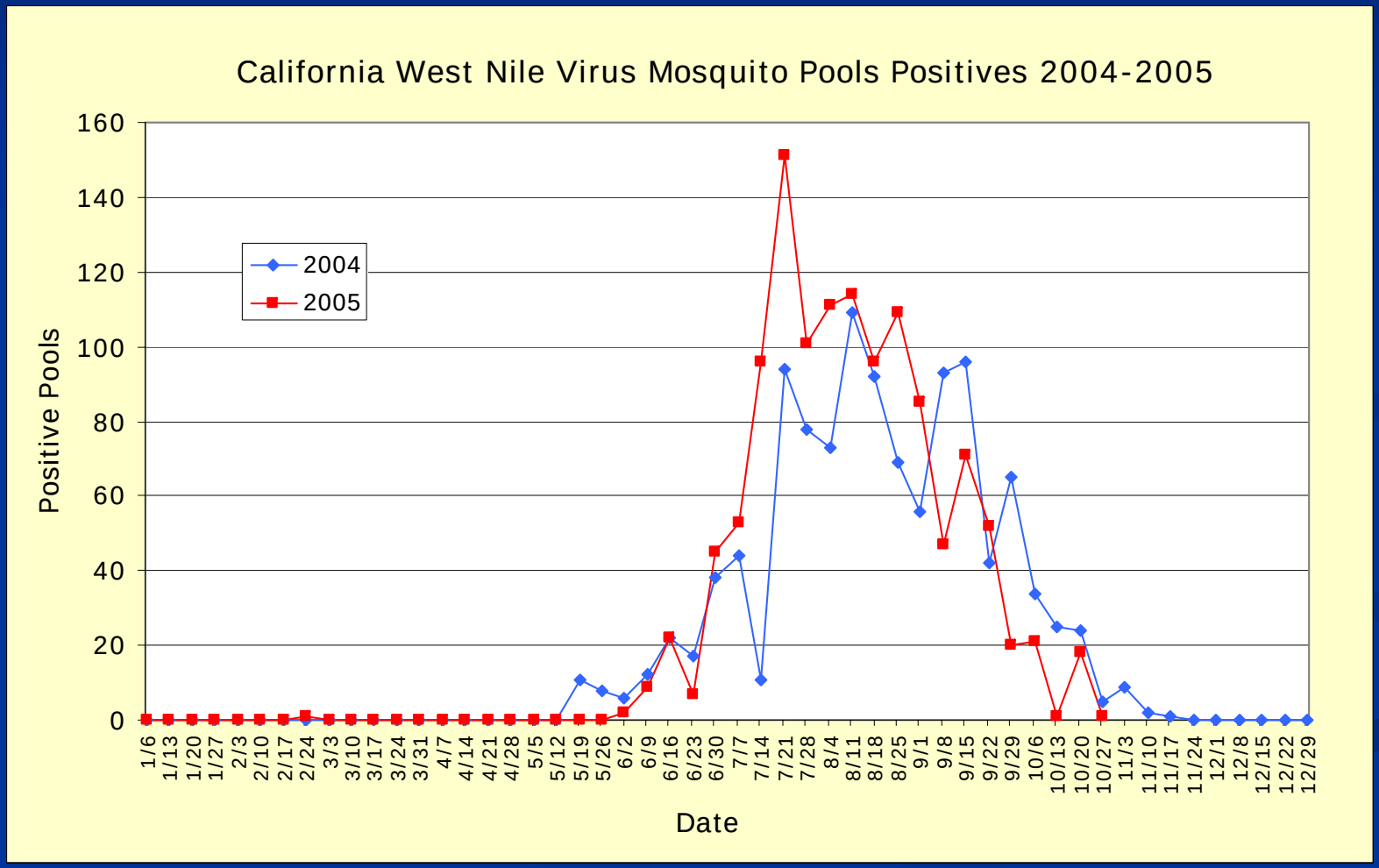


Cx. pipiens complex, summer 2005

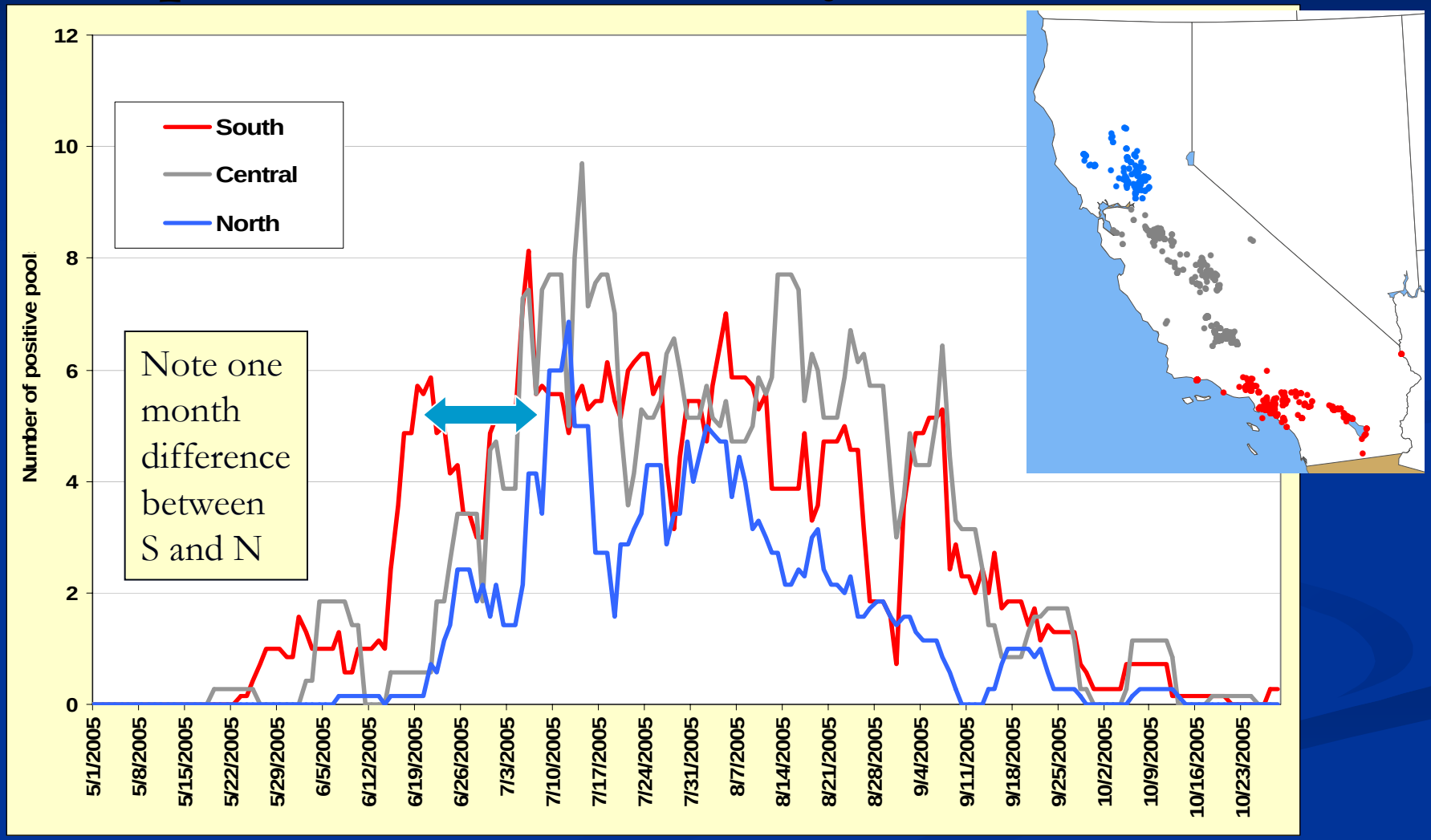
N/A – not available, mosquitoes tested locally



Seasonal occurrence of WNV positive pools in California, 2004 - 2005



Seasonal occurrence of WNV positive pools in California by latitude, 2005



* numbers shown are 7-day moving averages to smooth the counts for visualization.

Vertical transmission by *Culex*

Culex species	Pools	Total	WNV pos
F1 from host-seeking females or resting in Kern			
quinquefasciatus	83	3587	0
stigmatosoma	14	627	0
tarsalis	46	2151	6
thriambus	16	798	0
Reared from larvae in Coachella			
tarsalis	62	2506	0
Reared from larvae in Sac/Yolo			
tarsalis	276	12469	0
pipiens	40	1602	0

Collected as immatures or were the F1 progeny of field collected females reared in the lab, held until >3 d old and then tested for WNV by RT-PCR.

Comparison between RAMP and RT-PCR for field mosquito pools ground in RAMP buffer vs mosquito pool diluent

RAMP Buffer

		RT-PCR Pos	RT-PCR Neg	Total
RAMP	Pos	85	25	110
	Neg	1	318	319
	Tot	86	343	429

Mosquito pool diluent

		RT-PCR Pos	RT-PCR Neg	Total
RAMP	Pos	9	0	9
	Neg	8	314	322
	Tot	17	314	331

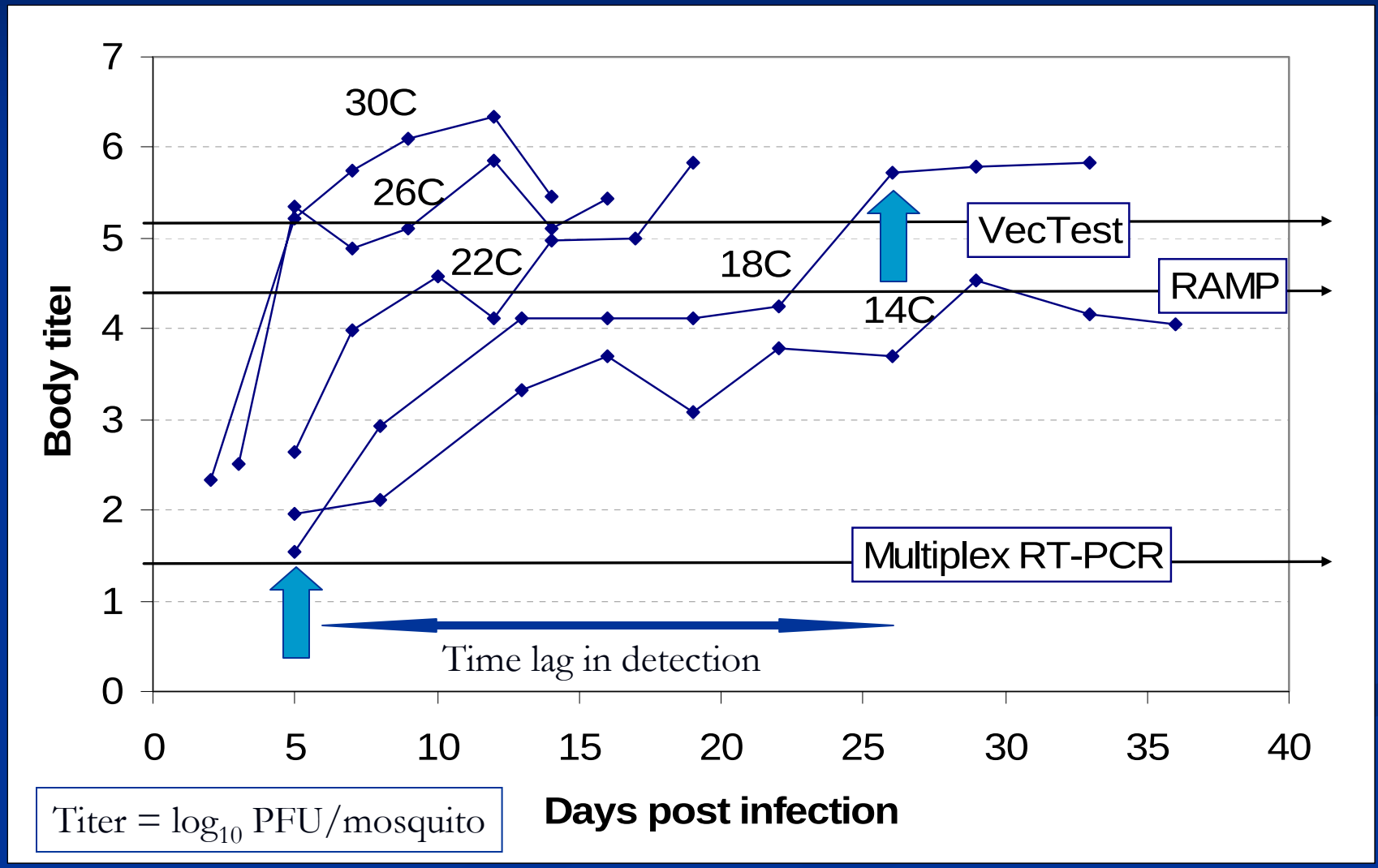
Disagreement: RAMP buffer degrades RNA limiting confirmation

Sensitivity lower for RAMP assay

Conclusions

- Antigen screening assays should be confirmed by more specific test
- Processing must be done so specimens are not compromised
- Decreasing recommended diluent volume can lead to false positivity
- RAMP sensitivity for mosquito pools 60-65% against multiplex-RT-PCR

WNV growth in *Cx. tarsalis* and detection by rapid Ag assays



Recommendations

- Sampling mosquitoes and testing for virus infection should be done in a systematic and consistent program using registered sites with all data submitted for incorporation into the state-wide program
- Testing from systematic sampling grid should be done by RT-PCR to provide:
  - Early detection of virus activity.
  - Comparable regional estimates.
- Emergency spot sampling during midsummer may be tested locally. Provides rapid determination if virus is being locally transmitted, but may underestimate how much transmission is occurring.

CVEC Arbovirus Laboratory Technical Staff

Not shown are Student Helpers: Kara, Thuan, Sean and David

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